L[illegible]OYD WISE, TREGEAR [illegible]CO

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OUR REF: MJPD/jmm/38013

YOUR REF:

31 October 1997

The European Patent Office,
Erhardstrasse 27,
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Dear Sirs

re: European Patent Application No: 92922300.6
(European Regional Phase of PCT/US92/09026
- Publication No: WO 93/08259) in the
joint names of NEW ENGLAND MEDICAL CENTER
HOSPITALS, INC and TUFTS UNIVERSITY
Our Folio: 38013

We refer to the Communication issued 22nd April 1997 pursuant to Article 96(2) and Rule 51(2) EPC and respectfully file herewith in triplicate new pages to replace pages 1 to 9 inclusive, 14, 15, 17, 20, 21 and 22 of the description, page 22 being cancelled, and all the pages of claims presently on file. Figure 2 of the drawings is also cancelled.

The invention is concerned with inhibitors to DP-IV and builds upon work involving the same inventors, or some of them, reported in each of the four documents relied upon by the Examiner.

We are pleased to note that the Examiner acknowledges that the applicants' claims as previously set out were entitled to

[illegible] present application is also to be found in D1 and in D2, this renders applicants' Claims 1 to 6 as previously recited lacking novelty.

The European Patent Office 31 October 1997

We respectfully disagree with the Examiner's conclusion.

Since the date of these publications which incorporate Table I, the present applicants have successfully separated the L,L-optical isomers from the L,D-optical isomers. As indicated, for example, for Pro-boroPro at the foot of page 20 of the present description (where an obvious correction has also been made), they have quite different inhibition constants, the L,L-isomer being significantly better than the L,D-isomer. Each of applicants' claims is limited to the L,L-isomers, none of which are disclosed in the cited art. Table I of D1, D2 and page 19 of the present application is essentially irrelevant to the L,L-isomer which had not been successfully separated and then compared with the L,D-isomer until the present invention.

For completeness, we should refer the Examiner to the "Supplementary Material" which appears at the end of document D2 and which, so far as the applicants are presently aware, may be said to represent the closest art.

There appears to be a suggestion at the third paragraph on page 3743 (left column) of separation of the L and D isomers. The passage in question is poorly reproduced in our copy and reads as follows:

> "Preparation of H-Ala-boroPro-pinacol. Boc-Ala-boroPro-pinacol was deblocked at 0°C with 3.5 molar excess of 4n HCl-Dioxane. Reaction was stirred at 0°C for 15 minutes and at room temperature for one hour. Flash-evaporation yielded a white foam. Purification was by silica gel chromatography with 20% methanol in ethyl acetate as the eluant. NMR analysis indicates that this column partially separates the two isomers of Ala-boroPro-pinacol. The early fraction appears from the NMR spectra to be approximately 95% enriched in a one isomer (sic). Because this early fraction has more inhibitory power than the later fractions at equal concentrations, we presume that early fraction is enriched in the L-boroPro isomer. These fractions were collected, precipitated with hexane to an extremely hydroscopic white powder. Further characterisation of the isomers based on stereospecific synthesis will be published in a

A better copy of D2 is provided for the Examiner's use.

Cont/d.....

Thus it will be seen that the "Supplementary Material" in D2 states that the authors had "presumed" that the L-isomer of H-Ala-boroPro had been purified to 95%. This presumption was *incorrect*, as is clearly apparent by comparison between the NMR data reported in D2 with the subsequently obtained NMR data for the optically pure L-isomer, as is explained in the enclosed letter from one of the inventors, Dr. Bachovchin, to our instructing United States attorneys.

It is well known in the art that NMR data is able to distinguish geometric (that is: *cis* versus *trans* isomers) as well as optical isomers (that is *L*- versus *D*-isomers). As Dr. Bachovchin explains, the only proper conclusion which can now be drawn is that the authors of D2 did not have the optically pure L-isomer. That being so, there is no lack of novelty. It is equally clear that D2 does not teach how to separate the L-isomer.

D2 describes the preparation of N-boc-Ala-boroPro-pinacol on page 3743 and reports that initial analysis of this preparation indicated that the sample was a "50/50 diastereomeric mixture" and that "attempts to resolve the isomers at this point were not successful". D2 also describes the preparation of H-Ala-boroPro-pinacol from the 50/50 diastereomeric mixture. As noted above, the preparation was accomplished by deblocking the amino terminal at 0°C with excess of HCL-dioxane and then allowing the reaction to continue at 0°C for 15 minutes, followed by *room temperature incubation for one hour*. Subsequent purification was attempted on a silica gel column with 20% methanol in ethyl acetate as eluant. Substantially this procedure was described on page 14 of the present patent application but has been excised because it is inaccurate.

The only description of any type of purification of the stereo isomers in D2 is found in the paragraph which we have quoted above. Although the authors of D2 presumed that the early fraction eluted from the silica gel column was the L-isomer of Ala-boroPro-pinacol, no support for this can be derived from the document itself or from any later document. It is true that at the time D2 was submitted for publication, the authors did think that the L-isomer was in the "early fraction". At that time they did not fully appreciate the multiple forms (for example: geometric isomers, optical isomers, and intramolecular reaction products) in which the dipeptides could exist. A further lengthy

Dipeptidyl Peptidase IV. A New Method for the Analysis of Slow, Tight-Binding Inhibition", Biochemistry 32: (1993) a copy of which is enclosed. This later publication describes the

preparation of L,L and L,D Pro-boroPro Diastereomers by C18 HPLC as described in the legend of Fig. 2 thereof. these conditions are substantially those of the HPLC separation conditions provided on page 20 of the present European patent application. It was these steps which were the key to separation of the L,L and L,D isomers.

The Biochemistry reference further illustrates the structure of *trans*-Pro-boroPro and shows its chiral centres in Figure 1. According to this later document, the coupling of L-pro with racemic LD-boroPro is expected to yield a mixture of two diastereomers: L-pro-L-boroPro and L-pro-D-boroPro. Figure 1 does not illustrate the potential *cis* form of these stereoisomers. Again, according to this later reference, the absolute configurations of the purified isomers were designated on the basis of a "detailed NMR study" (see page 3). At the time document D2 was submitted for publication, the authors had not performed a sufficiently detailed NMR analysis to determine, in fact, whether the two peaks eluted from the silica gel column represented the two optical isomers (diastereomers) of Ala-boroPro or whether the two peaks represented the *cis* and *trans* forms of one stereoisomer or a mixture of the *cis* and *trans* forms of the two different optical isomers. In the light of later work, it is clear that without the detailed NMR analysis, one simply could not reasonably conclude that the "early fraction" of the silica gel column described in D2 represented the L-isomer. In fact, the NMR data for the optically pure L-isomer when produced plainly shows that the "early fraction" referred to in document D2 was not the L-isomer. At the time that D2 was submitted for publication, the authors thereof did not appreciate that Ala-boroPro could undergo an intra molecular reaction to form a cyclic (and less active) intermediate. This undesired side reaction is in fact referred to at the foot of page 14 of the present European patent application.

It is to be noted that D2 describes conditions for preparing the H-Ala-boroPro which include the step of incubation at *room temperature for one hour* following the deblocking step. It is now appreciated that under these conditions, H-Ala-boroPro is likely to have undergone an intra molecular reaction to form a six-membered ring and, potentially, subsequent further reactions. Accordingly, the applicants now believe the "early" and "later" fractions referred to in D2 may well represent the linear and cyclic forms respectively, of the H-Ala-boroPro dipeptide without

chromatography altogether as a basis for purifying the stereoisomers. Instead, they have developed an alternative reverse phase technology (the C18 preparation described on page

20 of the present European application and in the 1993 Biochemistry reference referred to above) to separate the purified L-isomers.

In view of their later successful reverse phase technique, the applicants were not aware when the present application was filed precisely why it was that the silica gel separation technique had been unsuccessful but, as explained by Dr. Bachovchin, they do now know for sure that the "early" and "later" fractions which had been eluted from the silica gel column had not in fact represented a separation of the optical isomers.

Further discussion of the various isomers and of their NMR spectra can be found in Sudmeier *et al*, "Solution Structures of Active and Inactive Forms of the DP IV (CD26) Inhibitor Pro-boroPro Determined by NMR Spectroscopy", Biochemistry 33: (1994) and Sudmeier *et al*, "Solution Structures of the DP IV (CD26) Inhibitor Val-boroPro Determined by NMR Spectroscopy", Magnetic Resonance in Chemistry, Vol. 33, 959-970 (1995), copies of each of which are enclosed for the Examiner, and in each of which Dr. Bachovchin was a co-author.

Given the apparent promise of separation set out in D2, it would have taken other workers some time (as it did the present inventors) to appreciate that it was wrong and that the situation was more complex than had appeared at first sight. There was thus no motive or teaching which would at the time have directed the man of ordinary skills in this art to successful separation of the L and D-isomers, without which the present invention could not have been achieved. Accordingly, there plainly is inventive merit in applicants' claims as set out.

The Examiner will note that in addition to the boronates (Claim 1), the applicants also claim the corresponding phosphonates and fluoroalkyl ketones (Claim 3). As can readily be seen, the formula of Claim 3 can easily be derived as a sub-set of the formula:

"Group I-Group II"

where Group I has the structure set out at the top of page 3 of the PCT specification as published and Group II has the structure set out at line 9 of page 4 of the PCT specification as

We should also comment briefly upon the Examiner's reference to Article 83 EPC. Though much of the specification is written in terms of the production of boroprolines generally and with

particular reference to H-boroproline and H-Ala-boroPro, there is plainly disclosure of separation of the L and D-isomers and the indication that the L,L-isomers are significantly better inhibitors than the L,D-isomers. As a practical matter, the man of ordinary skills in this art will have no difficulty in making any of the inhibitors within the scope of applicants' claims on the basis of the teaching set out in the patent application. Other peptides besides the specific examples given can readily be produced by entirely analogous synthesis and the optical separation can then be carried out in exactly the same fashion as that explained in the patent specification for particular examples.

The applicants have endeavoured to deal comprehensively with all the matters arising in this case, but if the Examiner should have any query on any particular point or feel that some additional explanation would assist or if the Examiner feels that there is any point which has been overlooked, he is cordially invited to telephone the undersigned in the hope that this will assist in resolving the problem.

In the unlikely event that at this stage the Examiner decides that the application should be refused without further communication, but only in that case, the applicants respectfully request the appoint of Oral Proceedings. We trust, however, that no such proceedings will prove necessary in this case and that the examination procedure can be continued in writing. We further trust that in the light of this present submission, the Examiner will now be satisfied that the specification in its presently amended form meets the requirements of the European Patent Convention so that the next communication from the Examining Division may be that under Rule 51(4) EPC.

EPO Form 1037 is filed herewith in order that the usual acknowledgement of receipt may be issued.

Yours faithfully,

M. J. P. DEANS
Authorised Representative